

Mutational Analysis of Residue Asn348 in HIV-1 Reverse Transcriptase

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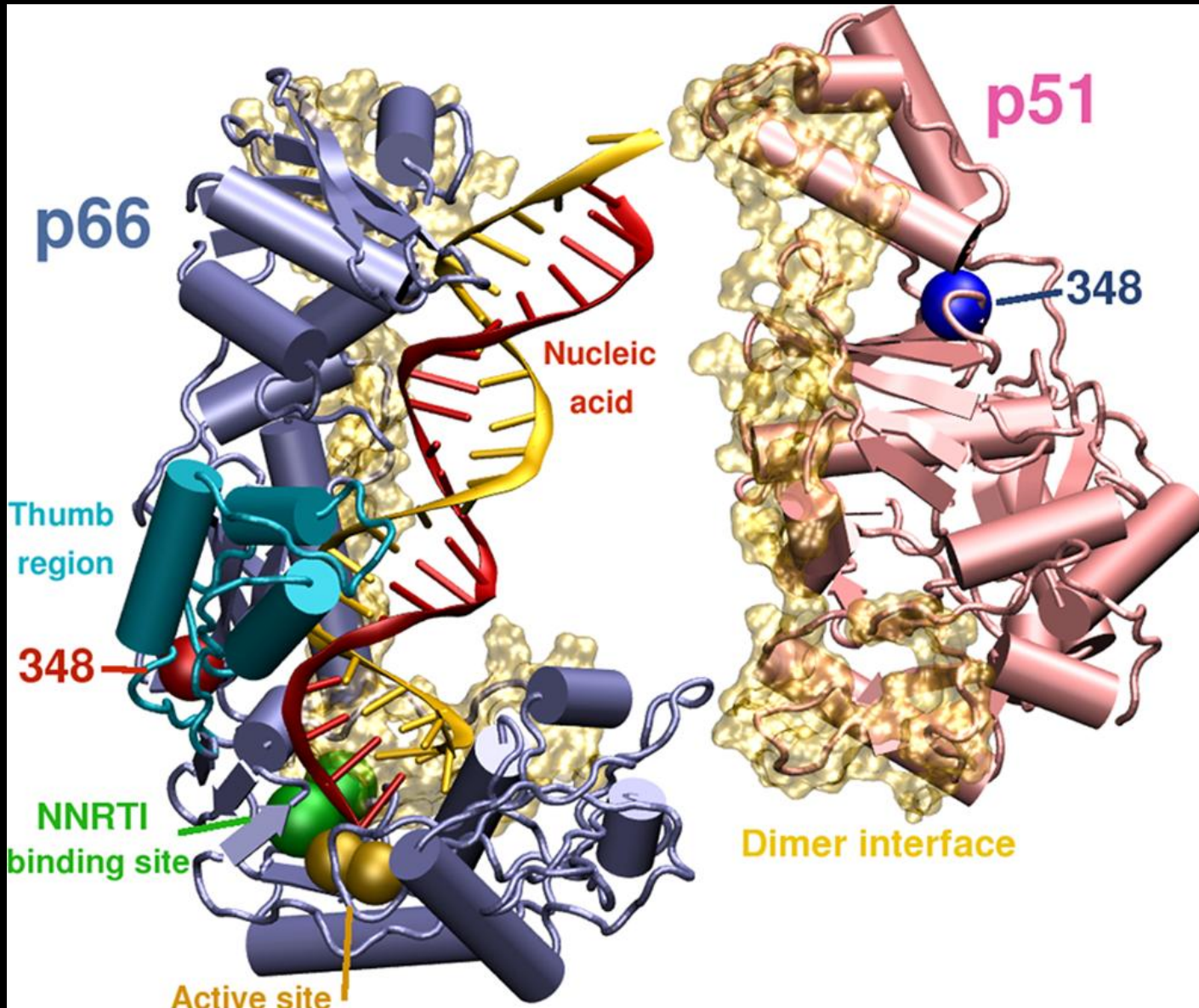
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Background

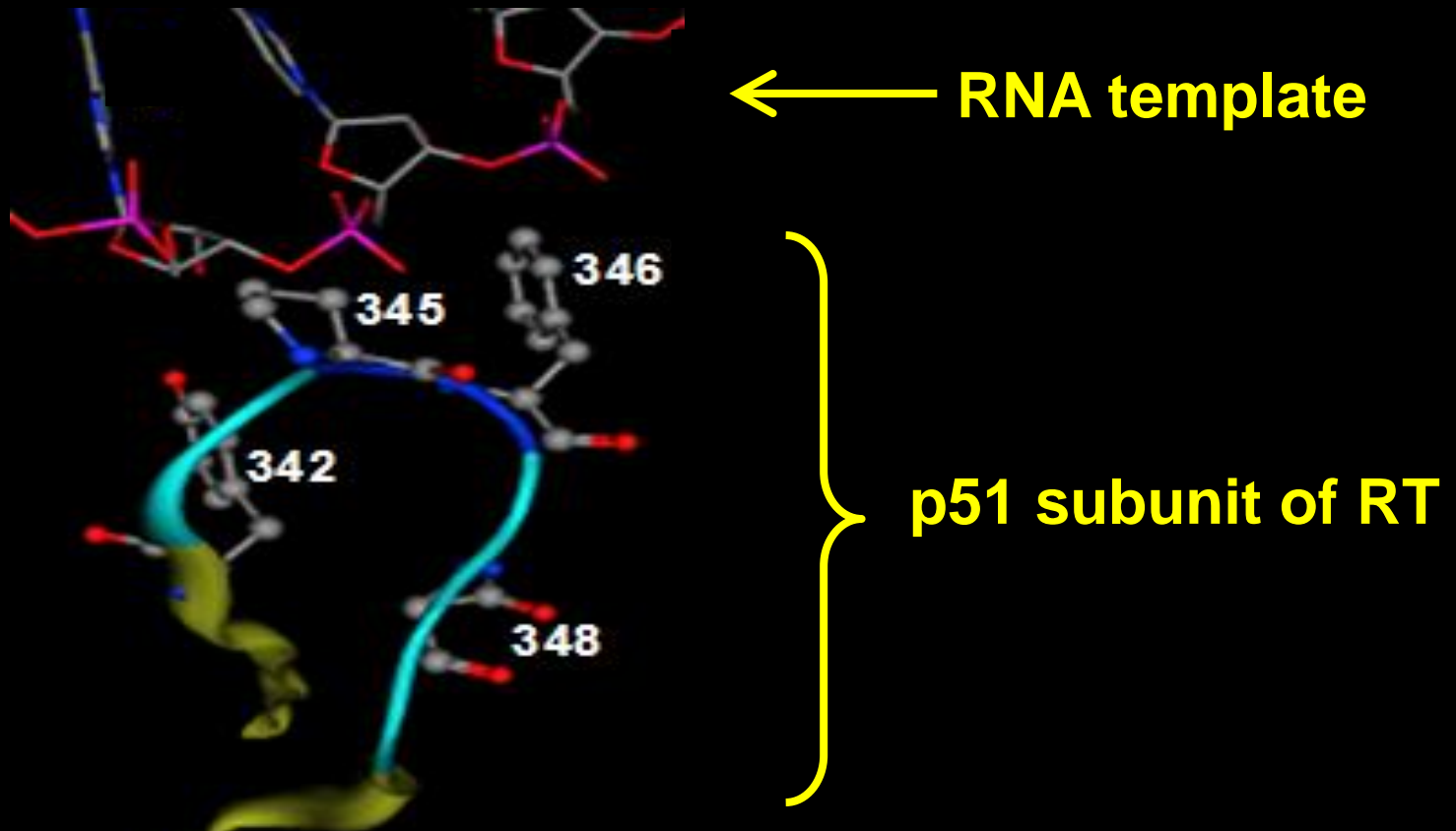
- N348I in HIV-1 reverse transcriptase (RT) confers zidovudine (AZT) and nevirapine resistance both at the virus and enzyme level (Yap *et al* PLoS Med 2007)
- Biochemical studies have suggested N348I in HIV-1 RT confers AZT resistance, in part, via an RNase H-dependent mechanism (Yap *et al* PLoS Med 2007; Ehteshami *et al.* JBC 2008; Delviks-Frankenberry *et al.* PNAS 2008)
- Mechanism for nevirapine resistance is not entirely clear...but studies by Biondi *et al* (CROI 2009) and Nikolenko *et al* (Abstract 31) address this

Structural basis of resistance?



No RT structure with RNA/DNA duplex that extends into RNase H active site

- Crystal structure of human RNase H1 complexed with an RNA/DNA hybrid (Nowotny et al. Mol Cell. 2007)
- Modeled RNA/DNA T/P duplex that extended into the RT RNase H domain



Molecular model of N348I in p51 subunit of RT



Loss of interaction:

N348I, N348L, N348E

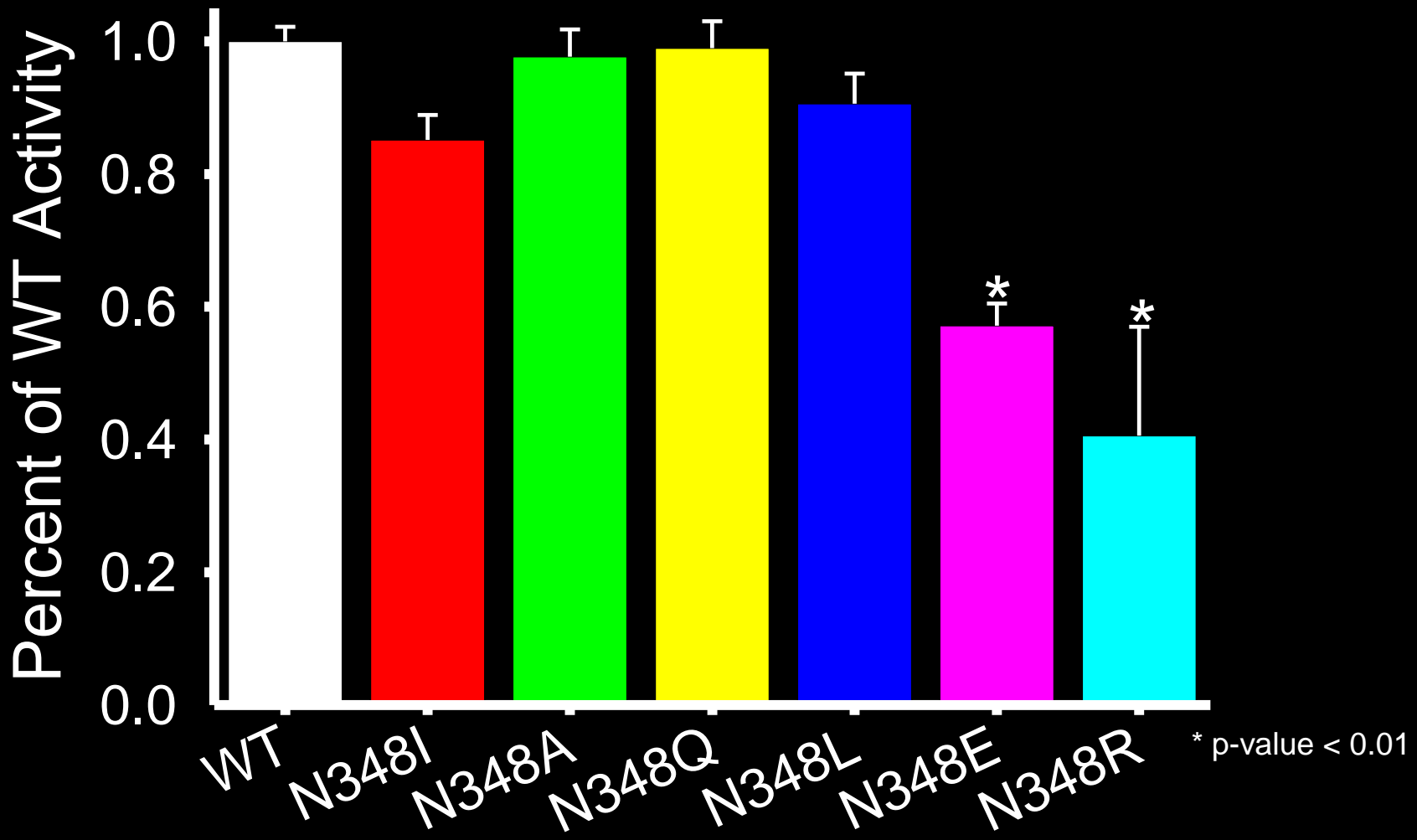
No impact:

N348A, N348Q

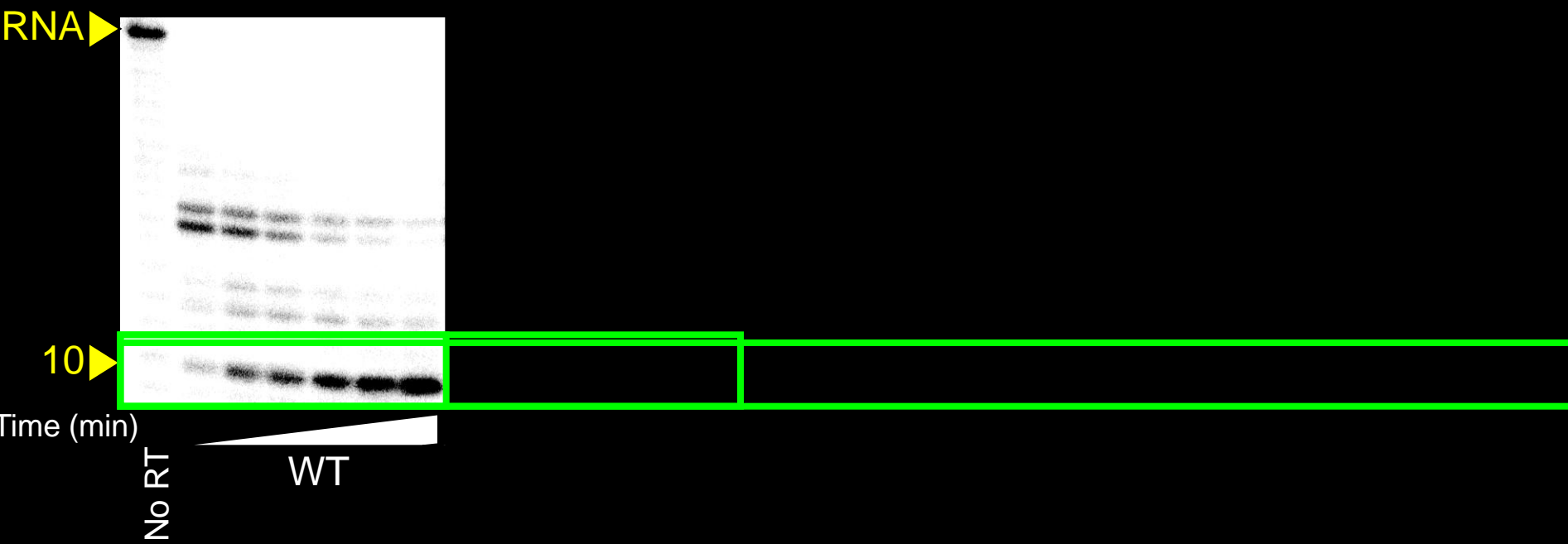
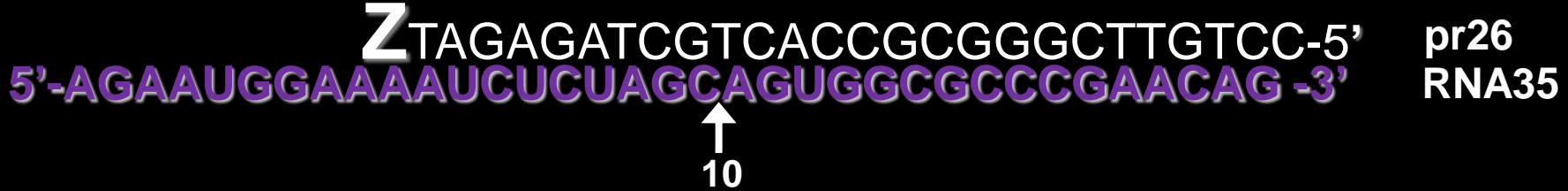
Enhanced interaction:

N348R

DNA polymerase activity of N348 mutant RTs



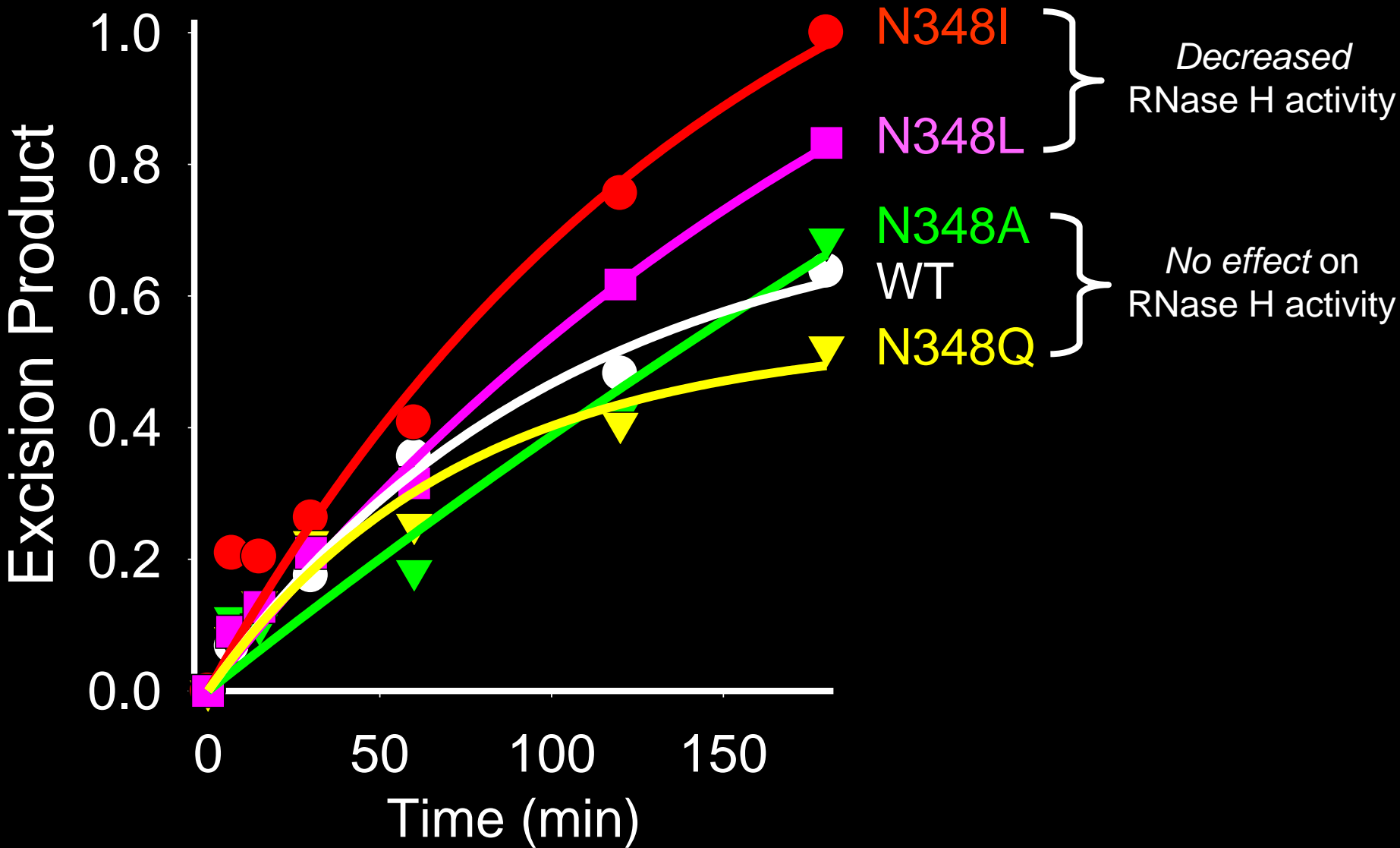
RNase H cleavage activity of N348 mutant RTs



Significantly decreased cleavage:
Similar to WT:

N348I; N348L
N348Q; N348A

AZT-MP excision activity of N348 mutant RTs



Nevirapine susceptibility of N348 mutant RTs

Enzyme	IC ₅₀ [nM]	Fold Change	p-value
WT	0.92 ± 0.18	--	--
N348I	3.31 ± 0.60	3.6	0.02
N348A	3.44 ± 0.19	3.7	< 0.01
N348Q	2.43 ± 0.29	2.6	0.05
N348L	2.84 ± 0.76	3.1	0.01

All N348 mutant RTs conferred similar nevirapine resistance!

Conclusions

- Mutations at residue N348 (e.g. I,L) that decreased RNase H cleavage increased RTs AZT-MP excision activity.
- Surprisingly, the relative RNase H/AZT-MP excision activity of mutant RTs correlated with the molecular models (mutations in both subunits of RT).
- Nevirapine resistance of N348 mutant RTs did not correlate with the RNase H/AZT-MP excision activity.
- These data suggest that the mechanisms underlying nevirapine and AZT resistance might be different!

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